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Original Articles

Diaphragm shortening and intrathoracic pressure during hypercapnia in rats

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The purpose of this study was to determine the relationship between intrathoracic pressure (ΔITP) and diaphragm shortening (DS) during the development of diaphragm fatigue. Fatigue of the diaphragm was produced by having rats breath 15% CO_2 in O_2. Diaphragm shortening increased significantly to 178% of control during the first 5 min of hypercapnia and then decreased to 86% of control at approximately 80 min. Twenty minutes after terminating hypercapnia, DS increased to 115% of the prehypercapnic value. ΔITP increased to 199% of control following 5 min of hypercapnia and continued to increase, reaching 267% of control at the end of the hypercapnic period. Twenty minutes later, ΔITP was 147% of control. These results illustrate that during increased respiratory work, DS can decrease while intrathoracic pressure remains increased. These findings suggest that intrathoracic pressure may not always reflect the contractile status of the diaphragm. These findings are consistent with other studies indicating that as the diaphragm fatigues, accessory respiratory muscle activity increases to maintain ΔITP.

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Introduction

There are thousands of patients in America that require mechanical ventilation. Weaning from mechanical ventilation requires accurate and reliable assessment of respiratory muscles for optimal patient outcome. Diaphragmatic fatigue is one of the most important causes of ventilatory failure because the diaphragm is the major inspiratory muscle (1) Many investigators use the change in transdiaphragmatic pressure (*P*di) during inspiration as an indicator of diaphragm fatigue. The change in *P*di during inspiration is determined not only by the degree of diaphragm shortening but also by the magnitude of shortening of the other inspiratory muscles. As diaphragm fatigue develops, shortening of other inspiratory muscles increases, resulting in there being no change in *P*di (2). Thus, it may be useful to develop a measurement of diaphragmatic activity. In order to accurately assess the development of diaphragm fatigue, a direct measurement of diaphragm force development or shortening is preferred.

Studies of diaphragm fatigue have concentrated on the isometric force development (3,4). Even in a fatigue state, the diaphragm normally develops only a small portion of its maximum isometric tension. Its principal function is to enlarge the chest cavity by shortening. Mardini and McCarter (5) used an *in vitro* rat diaphragm preparation to demonstrate that fatiguing the muscle, as defined by a decrease in isometric force development, was accompanied by a parallel decrease in diaphragm shortening. Based on these findings, they concluded that changes in shortening can also be a valid index of diaphragm fatigue.

It is generally accepted that muscle cell volume is constant throughout contraction and relaxation. Decreasing muscle cell length would be accompanied by an increase in cross-sectional area to maintain constancy. Poole and Mathieu-Costello (6) observed that the diaphragm sarcomere length decreased when lung volume was increased. In another study, Wait and Poole (7) observed an increase in diaphragm thickness of 50–80% when lung volume was increased from functional residual capacity (FRC) to total lung capacity (TLC).

The objective of this study was to determine the relationship between diaphragm shortening (measured by diaphragm thickness) and *P*di during the development of diaphragm fatigue. Hypercapnia was used to produce diaphragm fatigue. Hypercapnia stimulates respiratory drive through the medullary chemoreceptors (8). The elevated respiratory effort due to CO_2 exposure increases the work of breathing which leads to diaphragm fatigue (9). Several investigators suggest that the accompanying acidosis diminishes diaphragm performance by altering calcium metabolism (9,10). The negative inotropic effect of acute

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hypercapnia on the diaphragm is reversible with the restoration of normocapnia (10).

Methods

ANIMALS

Male Sprague-Dawley rats (300–400 g) were obtained from Sasco (Omaha, NE, U.S.A.) and allowed to acclimate for 48 h prior to experimentation. Standard rat chow and water were given *ad libitum*. The experimental protocol received approval from the Institutional Animal Care and Use Committee.

PREPARATION

The rats were anesthetised with an intraperitoneal injection of sodium pentobarbital (Anpro Pharmaceutical, 65 mg/Kg). Atropine (Elkins-Sinn, Inc., 0·4 mg/Kg) was administered intraperitoneally to reduce respiratory secretions. Anesthesia was maintained throughout the experiments by supplementing the initial dose of sodium pentobarbital every 30–45 min. Rectal temperature was continuously monitored and maintained between 36° and 37·5°C with a heating pad.

Rats were placed in the supine position and the trachea was cannulated using polyethylene (PE) 240 tubing. A miniature two-way non-rebreathing valve (Hans Rudolph no. 112754-2310; 0·5 ml dead space) was attached to the tracheotomy tube. A 4 Fr catheter with an inflatable balloon cuff was inserted in the oral cavity and advanced down the esophagus until the tip was approximately 1 cm above the diaphragm. The catheter was connected to a pressure transducer for continuous monitoring of esophageal pressure which served as a measure of intrathoracic pressure (ITP). The difference between ITP at end expiration and end inspiration thoracic pressure was defined as delta ITP (ΔITP). The magnitude of ΔITP was assumed to be directly proportional to the extent of shortening of the diaphragm and other inspiratory muscles.

A PE 50 catheter was inserted into the right common carotid artery and connected to a pressure transducer-recorder system for measuring systolic, diastolic and mean arterial pressure and heart rate. This catheter was also used for obtaining arterial blood samples for determination of pH, $PaCO_2$ and PaO_2.

The diaphragm was exposed via an abdominal midline incision. The abdomen remained open throughout the experiment resulting in the pressure on the inferior surface of the diaphragm being equal to atmospheric pressure. An ultrasound Doppler crystal (1 mm in diameter) was used for measuring dynamic diaphragm thickening as an index of diaphragm shortening (DS). The crystal was glued (Isodent, Ellman International, inc., Hewlett, NY, U.S.A.) to the inferior surface of the medial costal portion of the right hemidiaphragm. The crystal was coupled to a Crystal Biotech tracker and the output of the tracker was recorded on a strip chart recorder. The miniaturised crystal serves as a transmitter and receiver of pulsed Doppler ultrasound. Changes in diaphragm thickness were obtained by tracking the echo phases associated with a muscle layer moving through a referenced muscle plane. The reference plane was set at approximately the mid-level of the diaphragm and maintained in that plane throughout the experiment. Histological examination of the muscle fibers glued to the crystal for up to 4 h did not reveal any damage.

PROTOCOL

After approximately 30 min of stabilisation, baseline measurements (control) were obtained. Diaphragm shortening, ΔITP, respiratory rate and arterial blood pressure were obtained every 5 min by averaging these values during 10 breaths. A gas mixture of 15% CO_2 and 85% O_2 was given to the rats via the inspiratory port of the two-way non-rebreathing valve. Hypercapnia was maintained until DS decreased to 50% of the level obtained after 5 min of CO_2 exposure. This level of DS was defined as being diaphragm fatigue. Hypercapnia was then terminated and the animals given room air to breath for 20 min at which time post-control values for DS and ΔITP were obtained. The objective of the 20-min post-control period was to allow the diaphragm to recovery from fatigue.

Results

Figure 1 is an actual tracing of intrathoracic pressure, diaphragm shortening and arterial blood pressure during the prehypercapnic period (control). Diaphragm shortening which is measured as an increase in thickness is represented by an upward deflection in the tracing. With the onset of diaphragm shortening, the expected decrease in ITP occurs. Likewise, relaxation of diaphragm (return to baseline) results in an increase in ITP.

Diaphragm shortening excursions and ΔITP were measured and reported as a percentage of control. A representative experiment in which the rat is exposed to 15% CO_2 (hypercapnia) is illustrated in Fig. 2. After 5 min of CO_2 exposure, there was a marked increase in DS and ΔITP. Both DS and ΔITP remained elevated for approximately 80 min. Diaphragm shortening decreased over the next 20 min while ΔITP remained elevated. At this point in time, DS was 50% of DS following 5 min of hypercapnia. This 50% decrease in DS was defined as diaphragm fatigue and hypercapnia was terminated. After 20 min of recovery (breathing room air), ΔITP had decreased to control level, whereas DS had increased to greater than the prehypercapnic value.

Figure 3 summarises the DS results from 13 animals. Following 5 min of hypercapnia, DS was significantly increased to 178% of control. The mean time required for DS to decrease to 50% of the 5-min, hypercapnic value (diaphragm fatigue) was 79 ± 12 min. At this time, DS was 86% of the prehypercapnia (control) level. After a 20-min recovery period, DS was 115% of control.

Data for ΔITP are presented in Fig. 4 for the same 13 rats. After 5 min of CO_2 exposure, ΔITP was markedly increased to twice (199%) the control value. In contrast to

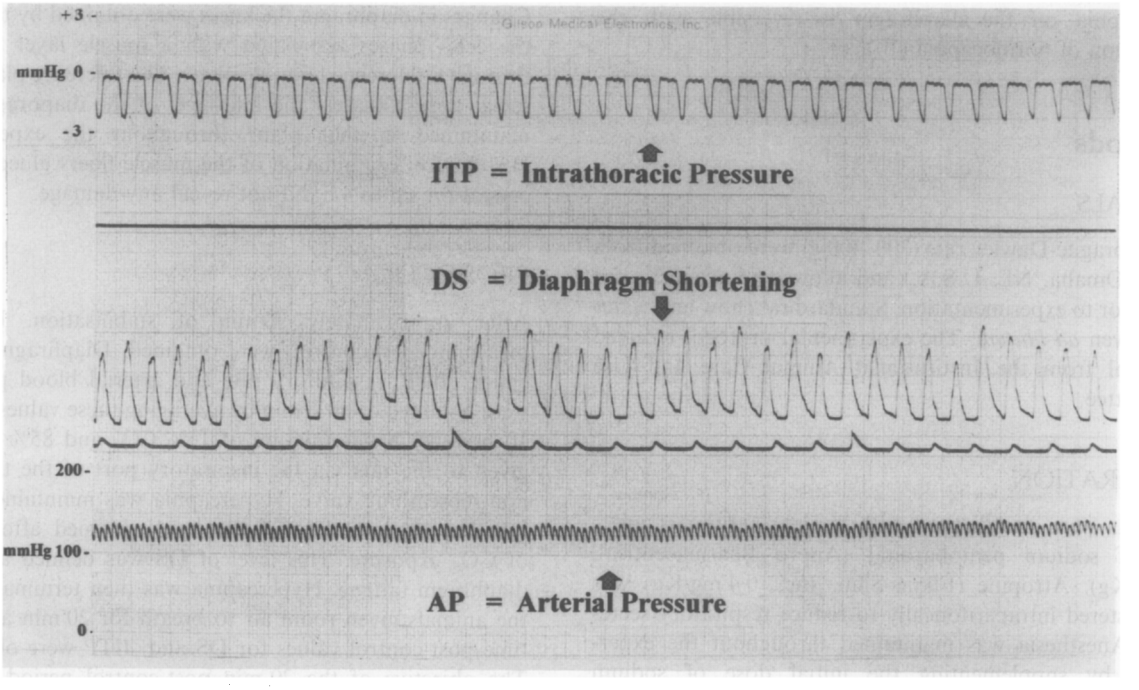

FIG. 1. Representative tracing of the intrathoracic pressure, diaphragm shortening and arterial pressure.

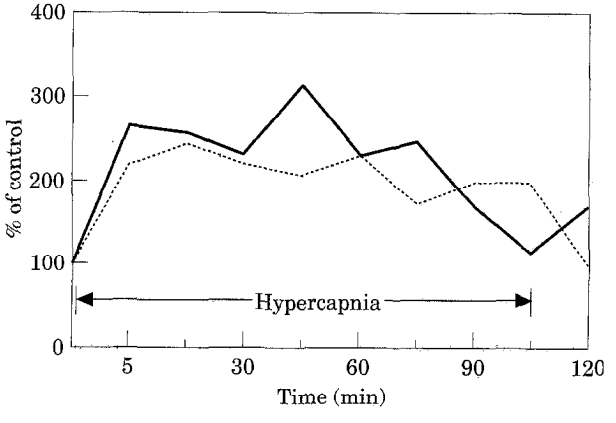

FIG. 2. Representation of diaphragm shortening (DS, ——) and Intrathoracic pressure (ΔITP, · · · ·) in a single rat.

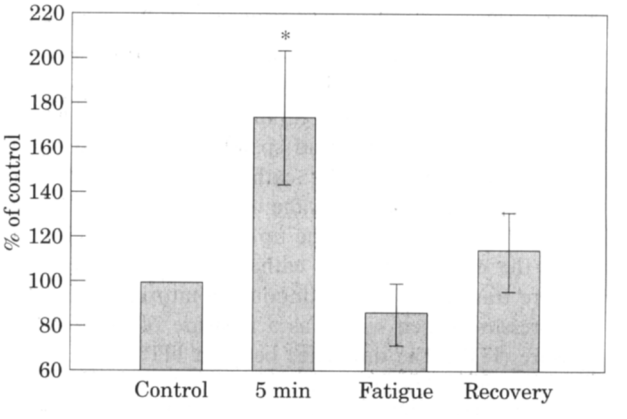

FIG. 3. Diaphragm shortening at control, 5 min of CO_2, fatigue and after 20 min of recovery (*P<0·05).

DS, ΔITP continued to increase throughout hypercapnia and was 267% of control at the time of fatigue. Following 20 min of recovery on room air, ΔITP was 147% of control.

Using a paired t-test, there was a significant difference between the control ΔITP and ΔITP at 5 min of hypercapnia (P=0·0258). Using a linear regression analysis, the change from control ΔITP to ΔITP at 5 min and at fatigue was significantly increased (P<0·0001 for both comparisons). Twenty minutes after terminating hypercapnia, ΔITP was not statistically different from the prehypercapnic value.

Discussion

This study indicates that diaphragm fatigue as measured by decreased diaphragm shortening can occur without a corresponding decrease in intrathoracic pressure. Exposure to 15% CO_2 increased diaphragmatic shortening and the difference between end inspiratory and end expiratory pressures (ΔITP). Following an average time of 79 min of hypercapnia, diaphragm shortening had decreased to 86% of the prehypercapnic levels while ΔITP remained increased. These findings could be the result of two factors: (a) a decrease in the resting length of the diaphragm; or (b) an increase in the activity of the accessory muscles of the rib cage. The end expiratory pressure was not changed throughout the experiment, suggesting that the resting length of the diaphragm was not significantly altered. The contribution of the accessory muscles to ΔITP was not assessed. It has been reported that hypercapnia increases the activity of the accessory muscles (2). This observation would account for ΔITP remaining increased when there was a reduction in the shortening capacity of the diaphragm.

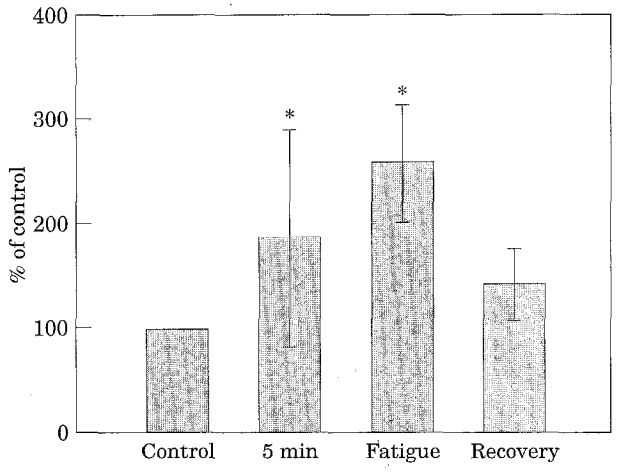

FIG. 4. Intrathoracic pressure at control, 5 min of CO_2, fatigue and after 20 min of recovery (*P<0·0001 using linear regression analysis).

This study elected to measure diaphragm shortening rather than diaphragm force development. There is presently not a miniaturised transducer for an *in vivo* rat model that would accurately measure force development of the diaphragm. For years, echocardiologists have used ultrasonics to measure changes in cardiac muscle thickness as an index of muscle shortening. In this study, a cardiovascular ultrasonic crystal for measuring changes in diaphragm was adapted as an index of diaphragm shortening. Recently, ultrasonics have been used to measure diaphragm shortening in humans (11,12). Mardini and McCarter (5) examined contractile properties of the rat diaphragm *in vitro*. They concluded that diaphragm fatigue can be defined in terms of both developed force and muscle shortening. Their study supported the present design to measure diaphragm shortening as an index of diaphragm performance. Other investigators have observed an increase in diaphragm thickness with increasing lung volume (7).

TRANSDIAPHRAGMATIC PRESSURE

The difference between abdominal pressure (Pab) and pleural pressure (Ppl) is called transdiaphragmatic pressure (Pdi). Although Pdi is frequently used to measure the force-generating capacity of the diaphragm, it is recognised that there is a considerable variability in Pdi values not related to the diaphragm (13). This study measured the difference between end inspiratory and end expiratory esophageal pressure (ΔITP) as an index of the total inspiratory effort. Since Pab in these experiments was always atmospheric, ΔITP is similar to Pdi. In this investigation, ΔITP remained elevated throughout the CO_2 exposure while DS decreased concurrently. These results are consistent with those of Yan *et al.* (2). They studied respiratory muscle contributions to intrathoracic and abdominal pressure changes before and following diaphragm fatigue induced by increased airway inspiratory resistance. Diaphragm fatigue was considered to have occurred when the subjects were unable to reach the target Pdi for three consecutive breaths. It was observed that the decrease in Pdi was not accompanied by a corresponding change in intrathoracic pressure. The authors concluded that this was due to an increase in activity of the inspiratory rib cage muscles during diaphragmatic fatigue. Grippi (14) stressed that during diaphragm fatigue, the contraction of the other inspiratory muscles can cause the diaphragm to move upward into the chest cavity while Pdi is increasing. This upward movement could be associated with an increase in diaphragm length. The present authors have observed decreases in thickness (i.e. lengthening) during inspiration in the severely fatigued diaphragm. It is believed that this is a result of the other inspiratory muscles 'stretching' the diaphragm. If active lengthening of the diaphragm does occur, it could be harmful to patients, particularly those weaning from mechanical ventilation.

DIAPHRAGM FATIGUE

Schnader *et al.* (9) measured abdominal pressure in anesthetised open chested dogs in which the abdomen was bound in a plaster cast. Abdominal pressure equaled Pdi produced by diaphragm contraction only. Diaphragm fatigue, defined as a 50% decrease in Pdi, was produced by repetitive phrenic stimulation while the animals were ventilated with various inspiratory gas mixtures of O_2 and CO_2. They observed that the diaphragm fatigue occurred sooner during hypercapnic than hypocapnic conditions. It was found that diaphragm fatigue was reversed by rest. The present observation that hypercapnia-induced diaphragm fatigue is reversed by 20 min of rest is in agreement with the findings of Schnader *et al.*

The results of this study support Roussos's (15) statement that diaphragm fatigue is an 'effort-dependent weakness'. In the present study, CO_2 exposure increased the work of breathing, evidenced by the increased ΔITP and a 12% increase in the respiratory rate. When hypercapnia was terminated, diaphragmatic shortening returned to the baseline levels within 20 min.

An important factor in diaphragm fatigue is the amount of chemical energy available and the washout of catabolites (4). Diaphragmatic blood flow is dependent upon arterial blood pressure and the degree of vasodilation. When the work of the diaphragm is increased, metabolic vasodilation occurs. If diaphragmatic work is further increased, there is a relative decrease in diaphragmatic blood flow due to mechanical compression of the blood vessels by the contracting diaphragm. If the work is decreased, there is an increase in blood flow during the early part of the recovery (16).

In the present study, it is possible that diaphragmatic blood flow increased because of hypercapnic vasodilation while arterial blood pressure remained unchanged. This coupled with the increase in arterial oxygen content (inspired O_2=85%) would result in enhanced oxygen delivery to the diaphragm.

The ultrasonic crystal used in this study was designed to measure shortening in cardiac muscle (17,18). With modifications, the ultrasonic crystal provides reliable data concerning the ability of the diaphragm to shorten. In

humans, there is no direct measure of diaphragm fatigue. Often patients who are ventilator-dependent have difficulty weaning because of the inability of clinicians to assess diaphragm fatigue. The ultrasound crystal used in this study may lead to a new method of assessing diaphragmatic function, particularly in those patients with respiratory difficulty. This crystal could be attached during thorax surgery or percutaneously via an abdomen method. It would be possible to isolate the signs of diaphragmatic fatigue more rapidly and accurately. This should result in better intervention with some patients with respiratory distress.

In conclusion, when rats experienced diaphragm fatigue, ΔITP remained elevated but DS decreased. These data suggest that intercostal and other accessory muscles take over the work of breathing to maintain adequate tidal volume when the diaphragm becomes fatigued.

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